



Bio-ethanol Production as Bio-solvent (Antifermenter), Antiseptic and Bio-fuel from Date Fruit Waste and its Suitable Properties Identification

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Authors' contributions

This work was carried out in collaboration between both authors. Author ABMSH designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors ABMSH and MSA managed the analyses of the study. Author MSA managed the literature searches. Both authors read and approved the final manuscript.

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ABSTRACT

Significance of the Study: Biomass derived bio-ethanol is biodegradable, nontoxic and suitable substitute for fossil fuels. It can be used as bio-solvent and antiseptic in the pharmaceutical industry and can also be used to reduce greenhouse gas emission like CO₂, CO, HC, SO_x and NO_x. Dates fruit biomass is an important subsistence crop in arid and semiarid regions of the world and a useful feedstock for bioethanol production. It is rich in monosaccharide and can be fermented easily.

Aims: The present study was conducted to investigate the effects of pH, temperatures, durations, yeast and enzyme concentration, water percent and parts of date fruit on bioethanol yield and its different properties.

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Methodology: Fermentation bioprocess was used to produce bioethanol from date fruit waste.

Results: The best yield (22.5%) of bioethanol in different parameters such as pH, temperatures, fermentation period and different yeast (*S. cerevisiae*) concentrations was found 5.8, 28°C, 5 days and 4 g/l, respectively. Moreover, viscosity and acid value were found within the limits prescribed by the latest American Standards for Testing Material (ASTM). There was no toxic elements found in the produced bioethanol and maintained the quality of ASTM standard. The elements found in bioethanol were Fe, Cu, Sn, Mn, Ag, Mo, Zn, P, Ca, Mg, Si and Na. The green house gas emissions such as hydrocarbons, sulfur dioxide, carbon dioxide, and nitrogen oxides were reduced while using the bioethanol, blended with pure fuel.

Conclusion: From the results it could be concluded that waste date fruit could be used as a potential feedstock for bioethanol production and suitable for 10% bioethanol as fuel for engine use without any modification of the engine.

Keywords: Rotten date fruit; bioethanol; greenhouse gas emission; engine performance.

1. INTRODUCTION

Nowadays, the global warming occurs by the use of excessive fossil fuels. Renewable bioenergy and biomass based fuels are required for replacing fossil fuel to reduce the greenhouse gas emission. Energy crisis may be happened due to the continuous increase of global petroleum prices which may impacts on human and social life [1]. In order to solve these problems, renewable energy should be developed and introduced new feedstocks. Bioethanol is a form of renewable energy that has been produced from common agricultural feedstocks such as sugar cane, potato, manioc and maize from the middle of last century. From 2007 to 2008, the share of bioethanol, which produced by fermentation process, has been increased from 3.7% to 5.4% [2]. New feedstock can be an innovative for bioethanol production as well as bioenergy regeneration.

A great deal of dates is however, thousands tones per year lost during the sorting, storage and conditioning. Some are fed to animals, but a lot of dates end up just rotting [3-5]. The non-use of this date for human food constitutes a real economic loss since it is rich in bioactive compounds and potential feedstock for bioethanol productions which can contribute to national economy to a great extent [6,7].

In addition, these clean energy sources have attracted the attention of researchers as alternative blending fuel due to their high octane number. Many researchers showed that the blending fuel showed better results in terms of fuel ratio, engine performance and exhaust emissions. This explains as the addition of ethanol to gasoline has two effects on the fuel blend properties: (1) an increase of the octane

number, (2) a decrease in the heating value [8-10]. They also reported that the CO and HC emissions decreased by 46.5% and 24.3% from starch-based feedstocks. In addition to that the best performance and emissions results were obtained for 20% ethanol with 80% gasoline blend. There is no literature found in this present research except few related literature. Therefore, the objectives the study were to investigate the effects of pH, temperatures, durations, yeast and enzyme concentration, water percent and different parts of date fruit on bioethanol yield and identify its different properties. In addition, to determine the engine emissions (CO₂, CO, HC, SO_x and NO_x).

2. MATERIALS AND METHODS

2.1 Raw Materials

Rotten date fruits (*Soccaria sp*) were obtained from local market, Jeddah, Saudi Arabia. The microorganisms employed were obtained from a commercial supermarket (commercial dry yeast [*S. cerevisiae*] and enzyme) [cellulose and amylase] and the ABO Laboratory, Kuala Lumpur, Malaysia.

2.2 Pretreatment

The collected samples were washed, cleaned and kept for 30 min in water bath at 40°C to make the fruit tissue soften. Then samples were took out and made it cool and finally prepared for fermentation process

2.3 Sample Preparation and Fermentation

Pulps were separated from seed and blended by blender. After blending properly the paste sample

was taken out to a jar. The 100 ml of pulp paste samples were kept in 500 ml schott bottle. The total soluble solid (TSS) of sample was measured before fermentation. The 1, 3, 5 gm/ l (w/v%) yeast were mixed with 15% water and incubated in the water bath at 40°C for 30 minutes to activate the yeast performance. In other parameter enzymes, combination of cellulase (v/v%) and amylase (v/v%) were used mixing with the yeast (w/v%) at the concentration of 0.5 and 1 g/l per enzyme with the seed waste (seeds were separated from flesh, seed coat and rest of the attached flesh to the seed) following the same procedure above mentioned. The total amount of enzyme was 1.5 g/l (cellulase + amylase + yeast = 0.5+0.5+0.5 g/l [0.5 per enzyme]) and 3 g/l (cellulase + amylase + yeast = 1.0 +1.0+1.0 g/l [1.0 g/l per enzyme]). The pH was adjusted 5.8 using 5 M sodium hydroxide (w/v%) (NaOH) to increase the pH and 1M acid hydrochloride (HCl) (v/v%) to decrease the pH and then shook well. The fermentation was conducted in the incubator and it was kept at 32°C for 5 days.

2.4. Fermentation in Different Variables

The fermentation method for different variables was same as stated above. Each fermentation was conducted in a 500 ml scott bottle sealed with a rubber stopper to ensure anaerobic conditions. In the different variables such as pH (5.6, 5.8 and 6), water percentage (20, 30, 40, 50 and 60%), yeast amount (2, 3, 4, 5, 7, 10 g/l), dates fruit part (pulp and seed), and temperatures (28, 32 and 40°C) were studied separately. The scott bottles having the samples were placed in the incubator (at 32°C, 4 g/l yeast and pH 5.8 in general parameters except temperature and pH parameters) for proper fermentation for maximum 5 days while being rotated at 130 rpm on a temperature controlled shaker. Samples were taken out from the incubator aseptically at 1, 3 and 5 days step by

step to measure bio-ethanol yield and assessed other parameters [9,10].

2.5 Filtration

After 5 days, the samples were taken out from the incubator and filtrated by clean folded cheese cloth. The samples were filtrated by using filter paper and left the samples for 2 hours until the raw bioethanol was settle down as clear solution without any residue. Then the raw bioethanol was distilled by vacuum evaporator to purify the bioethanol at 70°C of water bath. The bioethanol yield was measured by using spectrophotometer at 575 nm and compared with the standard curve [11].

2.6 Chemical Analysis and Engine Test

Bioethanol yield was determined by the Measurement of ethanol absorbance at 575 nm after completion the ethanol Assay reagent step using spectrophotometers and compared to the ethanol standard graph and calculated the percentage of bioethanol [11]. In the experimental study, viscosity, acid value and metal content were analyzed by multi element oil analyzer (MOA II). Three types of combination of fuel and bioethanol were used in this study. The first one was pure petrol fuel (called E0). The second one was bioethanol blended with petrol fuel containing 5% bioethanol (E5) and the third one was bioethanol blended with petrol fuel containing 10% bioethanol (E10). The multi cylinder hydra spark ignition engine with injection system was used. The tests were performed at 2000 rpm and the test fuels were gasoline (E0) and gasoline ethanol blends E5 and E10, the numbers following E indicate percentage of volumetric amount of ethanol. Fuel consumption was measured using Ohaus GT 8000 model (Gen 2 proton, made by Malaysia) and exhaust emissions were measured using Sun MGA 1200 model emission tester.

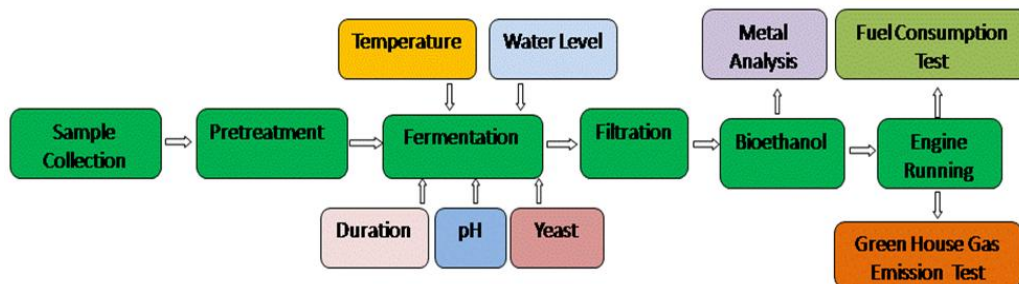


Fig. 1. Flow chart of whole bioethanol production and engine test at a glance

Flow chart of bioethanol production and engine test was shown in Fig. 1.

2.7 Statistical Analysis

Statistical analysis was completed by SPSS software. Significant difference among the treatments was evaluated by LSD ($p=0.05$) was calculated using the error mean squares.

3. RESULTS AND DISCUSSION

3.1 Effect of Fermentation Period

Fig. 2 showed that the bioethanol yield gradually increased along with the increase of duration and reached a maximum (22.5%) stage at 5th day. The results of the present research work showed the contribution of selecting the right variables such as pH, duration and temperature on fermentation progress. These variables can seriously affect the results of the reaction process and the amount of bioethanol yield through the hydrolysis and fermentation [12]. The bioethanol production in fermenting process depends on duration of fermentation and longer duration increases the fermentability of the hydrolyzates. After 5 days of fermentation, bioethanol content reached the maximal bioethanol yield (22.5%) [13].

3.2 Correlation of pH and Bioethanol Production

The effect of different pH parameters on bioethanol production has been shown in Fig. 3. The bioethanol yield increased gradually with increase the pH from 5.6 to 5.8 and decreased at pH 6. It was reported that the effect of pH on ethanol production from mahula flowers and found the maximum ethanol yield and fermentation efficiency at pH 5.5 [14].

It was reported that pH 6 showed higher bioethanol yield compared to pH 4 and 5 from pineapple waste feedstock [15]. It was observed that fermentation occurred at $pH > 6$, $pH 5.5$ and $pH < 4.5$. Sometimes it may depend on the feedstock which is used in the fermentation [16]. It was reported that pH at the concentration of 5.5, 5.8, 6 g/l was used in the fermentation and found best result at 5.8 g/l [17]. It was stated that a culture of *Candida lipolytica* was isolated and the microbe was used to study the production of lipase. The optimum pH, medium, and temperature for lipase synthesis were established using pH 5.8, 6 and 6.5 [18]. In addition, it was reported that the initial pH affected the levels of the alcohols production [19]. As shown in Fig. 3, the maximum specific bioethanol production (22.5%) occurred at pH 5.8. This indicated that the yeast was better adapted to utilize the substrate at pH of 5.8.

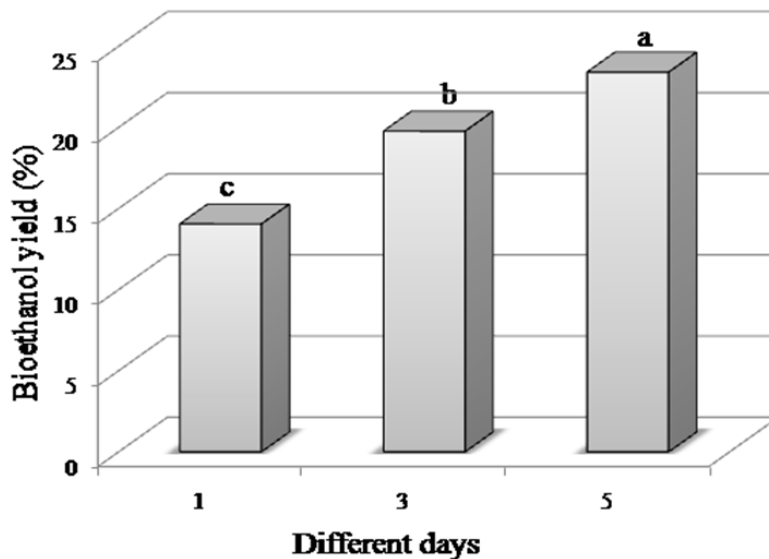


Fig. 2. Determination of bioethanol yield after fermentation at different days. Different letters mean the significance by LSD ($p=0.05$)

3.3 Effect of Temperature

Fig. 4 showed that bioethanol production decreased with the increase of temperature and maximum yield (22.5%) was observed at 28°C. This is probably due to the increase in cell number with the temperature range between 25-30°C. It was referred that the bioethanol concentration, bioethanol productivity and fermentation efficiency increased with the increase in fermentation temperature 25-30°C and decreased gradually between 30 and 35°C and drastically above 35°C [14,20].

3.4 Suitable Temperature Range for Fermentation

The best temperature for yeast growth has been reported to be closed to 30°C [21]. In this present study, the most effective temperature to increase bioethanol production was 28°C due to the more active yeast cells and greater activity of the fermentation pathway. The rise in temperature of fermentation may affect the composition of bioethanol by causing some loss in alcohol or low conversion of sugar.

3.5 Effect of Yeast Biomass Concentration

Data presented in Fig. 5 showed that as the concentration of yeast increased, the yield of

bioethanol increased up to 4 g/L and then decreased above this (Fig. 5). It was reported that there was a limit of yeast content above which yeast cells may not function to produce ethanol [22]. Another reason might be due to the decrease in porosity, lower oxygen interaction and low aeration inside the solution. It was observed that wine fermentation from grape and apple was a complex biochemical process during which yeasts metabolized sugars and other constituents as substrates for their growth by converting them into ethanol ranging from 2-3 g/l of yeast used [23]. Ethanol production was performed using 4 g/l yeast in the fermentation process [24]. It was reported that *Saccharomyces cerevisiae* as the ethanologenic strain in separate hydrolysis and fermentation was able to achieve 191.5 g ethanol/kg from corn stover using 5 g/l yeast concentration. A 5 g/l yeast concentration showed higher bioethanol yield compared to 1 and 3 g/l from pineapple waste feedstock [15].

3.6 Effect of Water Percentage

The difference of bioethanol yield at different treatments of water percent were evaluated in Fig. 6. It was reported that the production of bioethanol was improved due to the higher amount of water percent. There was no fermentation occurred at all in the case of 20 and 30% water. The linear correlation showed the positive effect.

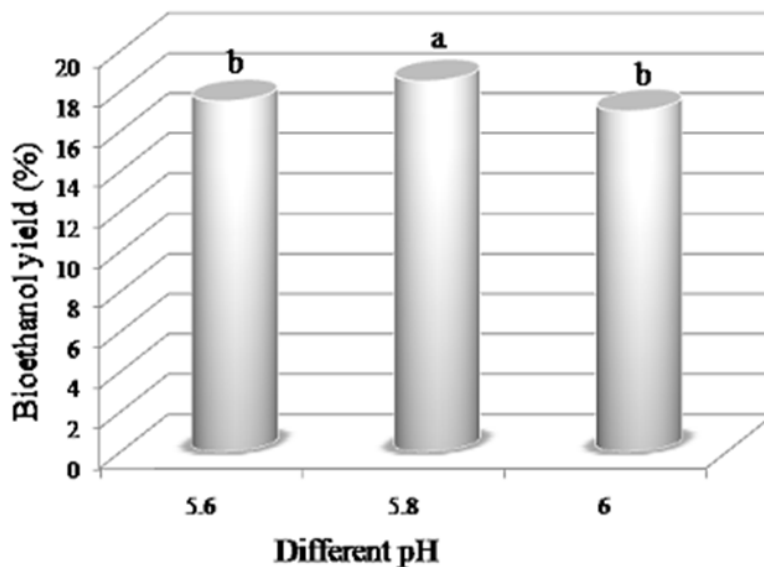


Fig. 3. Determination of bioethanol yield after fermentation at different pH. Different letters mean the significance by LSD (p=0.05)

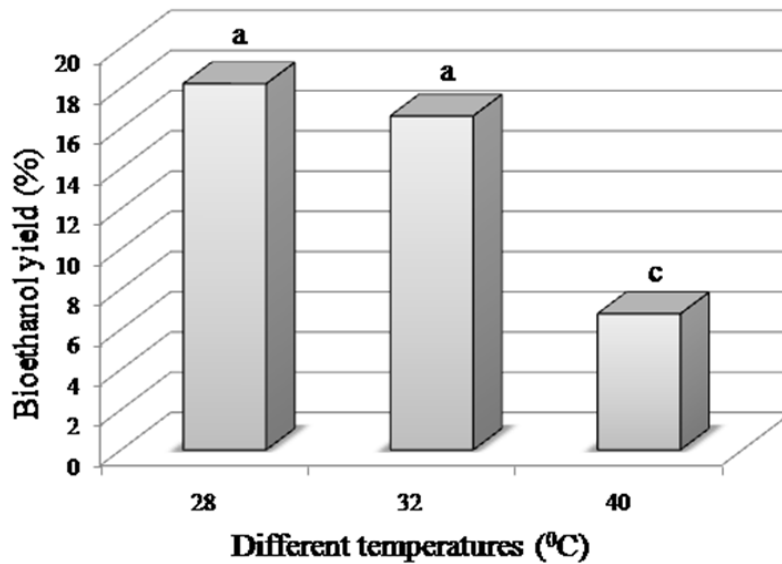


Fig. 4. Determination of bioethanol yield during fermentation at different temperatures. Different letters mean the significance by LSD ($p=0.05$)

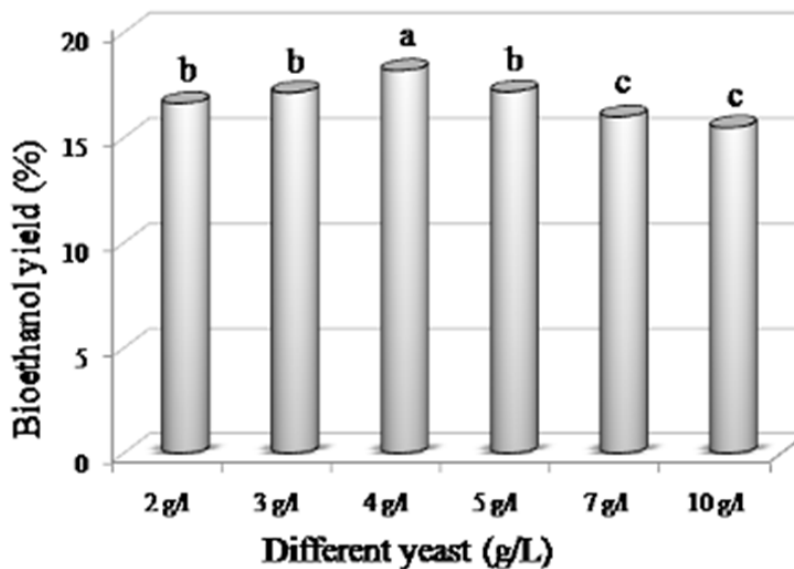


Fig. 5. Optimization of bioethanol yield at different yeast concentrations. Different letters mean the significance by LSD ($p=0.05$)

3.7 Effect of Different Enzymes

The yield of bioethanol from seed waste having different concentration of enzymes (cellulase and amylase) and yeast was shown in Fig. 7. The effect of 0.5 g/l per enzyme (Total of 1.5 g/l [cellulase + yeast+ amylase = 1.5 g/l]) treatment on the bioethanol yield was observed higher than that of 1.0 g/l per enzyme (Total of 3.0 g/l

[cellulase + yeast + amylase = 1.0+1.0+1.0 = 3.0 g/l]).

3.8 Engine Performance and Gas Emissions

In this study E5 and E10 were used in the ordinary petrol engine without engine modification. It is necessary to modify the engine

if bioethanol is used more blends (E15, E20 to E85) than E10. Bioethanol can be used in different blends to fuel vehicles. It was reported that E85 was used in ethanol based vesicle fuel with having engine modification low blends in petrol (E5, E10 and E15) were used without having engine modification. Modified vehicles are required for high bioethanol blends while not required in low bioethanol blends. High blends contain a high proportion of bioethanol and effectively substitute fossil fuels [25]. It was stated that E85 was a mixture of 85% ethanol and 15% gasoline, and was generally the highest ethanol fuel mixture found in the United States and several European countries, particularly in Sweden as this blend was the standard fuel for flexible-fuel vehicles [26]. Thailand introduced E20 in 2008 and E20 demand increased rapidly due to the most vehicle models launched. E20 is compatible and sales of E20 are expected to grow faster once more local automakers start producing small E20-compatible fuel-efficient cars [27].

E95 (96.5% hydrous bioethanol, 3.5% additives) was used in bioethanol buses, converted diesel vehicles and dedicated heavy diesel vehicles, such as waste collection trucks. E100 (100% hydrous bioethanol) was used in modified petrol

engine in Nanyang and petrol cars in Brazil [25]. Blending bioethanol into vehicle fuels had been enacted at the national level, USA with most mandates requiring a blend of 10% ethanol with gasoline without engine modification [28]. It was reported that E95 designated a blend of 95% ethanol and 5% ignition improver and was used in modified diesel engines where high compression was used to ignite the fuel as opposed to the operation of gasoline engines where spark plugs were used [29]. The diesel engine run on ethanol had also a higher compression ratio and an adapted fuel system [30]. It was reported that E100 was pure ethanol fuel and more recently used for flexible-fuel vehicles [28,31,32].

Figs. 8 and 9 showed the emitted smoke concentration. It was observed that the emission was significantly lower blended ethanol-gasoline fuel (E5% and E10%) than that of 100% gasoline. In respect of SO_x, NO_x and HC, the lowest emission was found in 10% ethanol+90% gasoline. On the other hand, decreasing ratio of CO emission was found to be higher than that of CO₂ emissions in 100% gasoline. Fuel consumption was decreased when the fuels contained higher amount of bioethanol, 10% (E10).

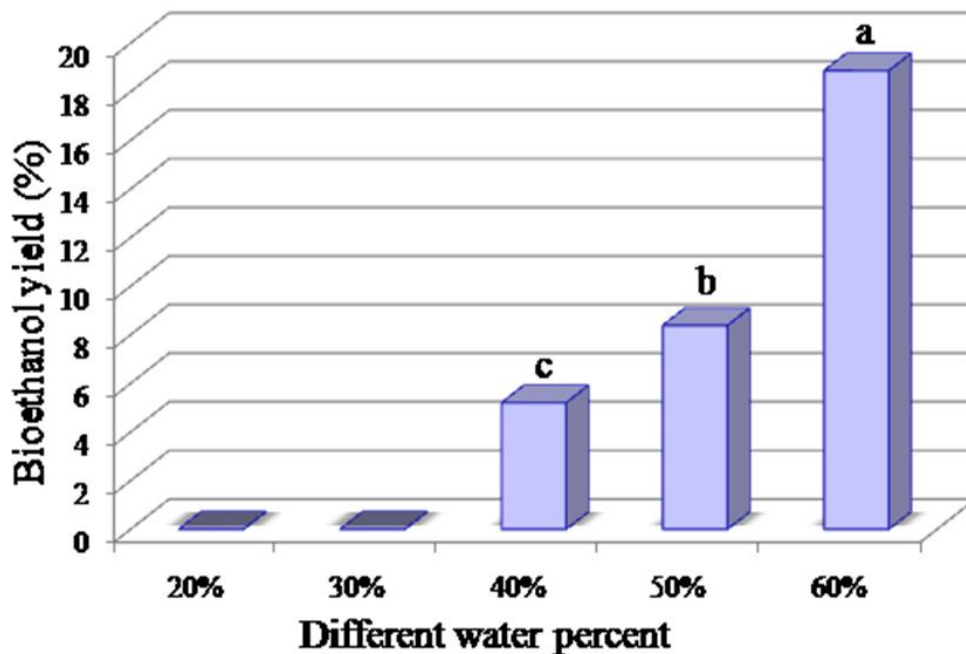


Fig. 6. Determination of bioethanol yield at different water percent. Different letters mean the significance by LSD (p=0.05)

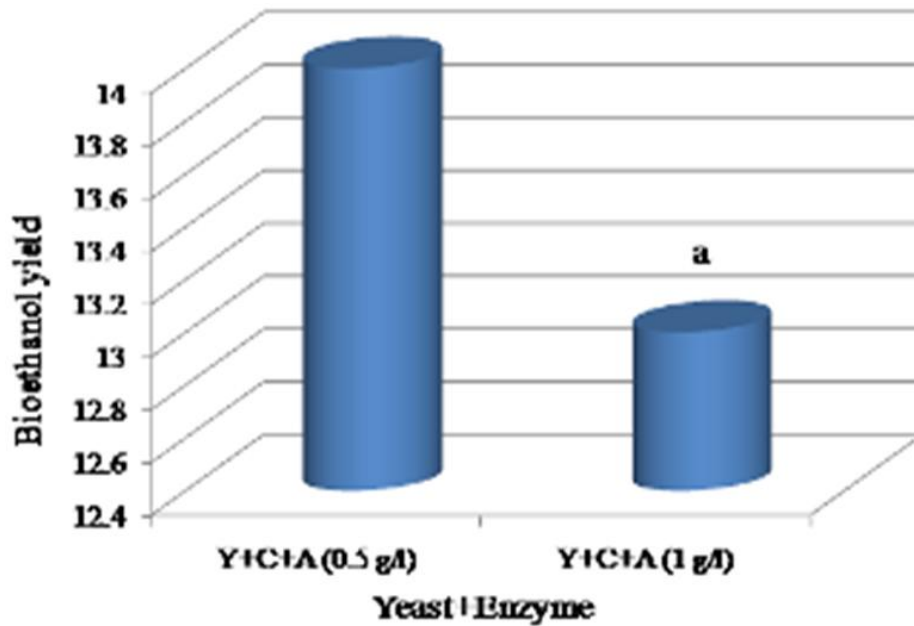


Fig. 7. Bioethanol yield at different yeast and enzyme concentrations. (Total of 1.5 g/l [cellulase + yeast+ amylase = 1.5 g/l] 0.5 g/l per enzyme, (Total of 3.0 g/l [cellulase + yeast + amylase = 1.0+1.0+1.0 = 3.0 g/l]) 1.0 g/l per enzyme. Different letters mean the significance by LSD (p=0.05)

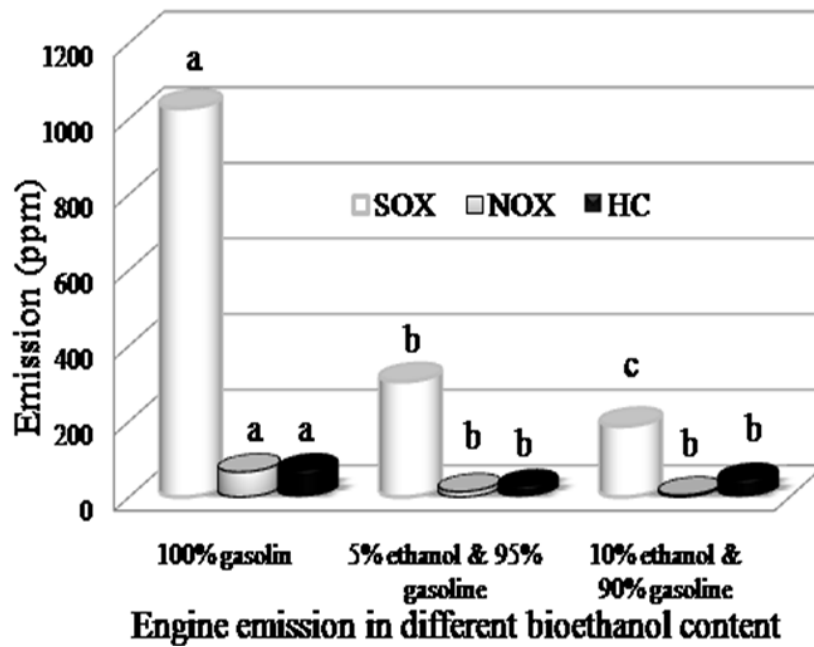


Fig. 8. Estimation of engine emissions (SOx, NOx, and HC) using 2 stroke engine at pure gasoline (100%), 5% bioethanol and 10% bioethanol. Different letters mean the significance by LSD (p=0.05)

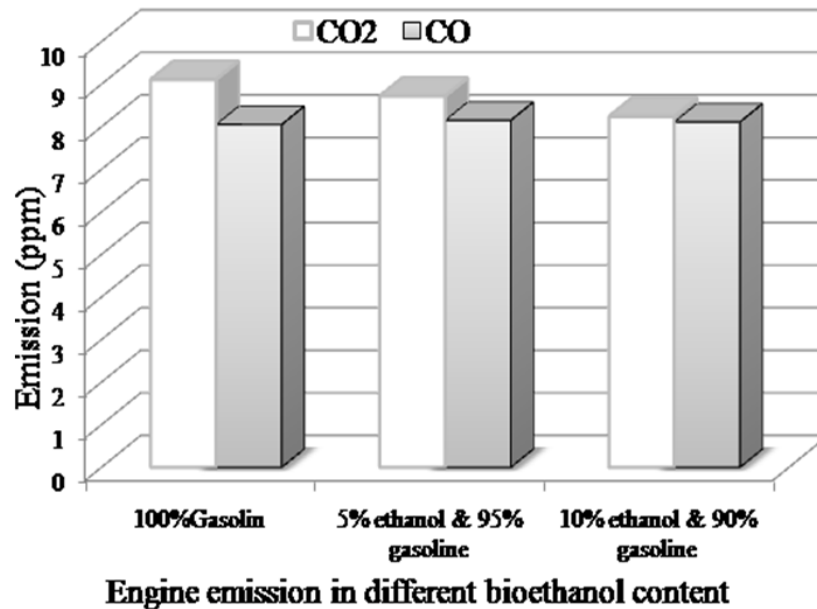


Fig. 9. Estimation of engine emissions (CO₂ and CO) using 2 stroke engine at pure gasoline (100%), 5% bioethanol and 10% bioethanol

It was reported that bioethanol could be blended with petrol or used as neat alcohol in dedicated engines due to the higher octane number, low cetane number and higher heat of vaporization and vehicle can diminish greenhouse gas emissions by 41–61% km⁻¹ driven, compared to gasoline-fuel vehicles [25]. The effects of different volumetric percentages of bioethanol–gasoline blends, ranging 0%, 5% and 10%, on engine emissions were tested on local engine (Gen-2 proton engine). The present study showed that the variations of the NO_x, CO₂, CO and HC emissions depended on the blending ratio at 2000 rpm engine speeds. Fig. 9 showed that 100% gasoline produced higher carbon dioxide and carbon monoxide engine emissions than the 5% ethanol + 95% gasoline and 10% ethanol + 90% gasoline blend throughout the experiment [33]. Bioethanol can be a viable transport fuel and reduces emissions of fossil carbon dioxide (CO₂). Bioethanol is biodegradable and less toxic and explosive than petrol. A range of fuel blends (E5, E10 and E15) can be produced from bioethanol. High blends (E85) can substantially reduce greenhouse gas emissions, depending on how they are produced. Due to the difference in properties between fossil fuels and bioethanol, high blends require some modifications to the vehicle engine and a dedicated fuelling infrastructure [29]. The E85 limit in the ethanol content was set to reduce ethanol emissions at low temperatures [29].

The higher oxygen content in the blending fuel favors conversion of the CO produced during combustion into CO₂. Fig. 8 showed that 5% ethanol + 95% gasoline produces higher NO_x emissions than the 10% ethanol + 90% blend for most of the speed range investigated. That was due to the faster flame speed and higher peak temperature in the combustion chamber of 5% ethanol + 95% gasoline. As it is well known that the NO_x formation is a strong function of peak chamber temperature. Blending gasoline also appears to be a good choice for HC emissions reduction, as shown by Fig. 8. The chemical structure of the ethanol-gasoline blend, with higher presence of carbon and hydrogen is less favorable for HC formation than 100% gasoline. Fig. 10 showed the lower fuel consumption in E5 and E10 than that of 100% gasoline. Considerable fuel consumption was decreased when the fuels contained higher amount of bioethanol like E5 and E10. The results taken from the present experiment referred that the engine performance and emissions were improved.

3.9 Presence of Trace Elements, Viscosity and Acid Value in Bioethanol

From the results in Fig. 11 demonstrated that the samples did not contain the toxic elements based

on American Society for Testing and Materials (ASTM) D4806 and ASTM D5709 standards. Table 1 showed the viscosity and acid value at different days. Both the properties maintained ASTM standard as well. The presence of elements Zn and Ca gave benefit because of these compounds provide an alkaline reserve to neutralize acidic by-products of combustion so thus can reduce the formation of insoluble compound and avoid corrosion [34]. The presence of trace elements such as Zn, P, Ca, and Mg in bioethanol solution due to the feedstock contains these in nature. According to the many researcher experiments, it was stated that the trace metals that contains in fruit juices consist of Pb (0.009 ppm), Mn (15 ppm), Ni (0.29 ppm), Zn (0.90 ppm) and Sn (0.45 ppm) [35]. Overall, the anhydrous bioethanol samples that have been produced from date wastes were safe to be used as one of the sources of fuel because they did not contain any toxic metal elements and some elements presence were in the range of limit acceptance based on the ASTM standard. In addition, both the viscosity and acid value at different days were maintained

ASTM D4806 and ASTM D5798 standard too (Table 1).

3.10 Bioethanol as Solvent and Antiseptic Use

From the Fig. 12, it has been seen that glucose content was started to reduce in the first (after 12 hours) and bioethanol was started to produce and made rotten the juice faster in the grape juice without produced bioethanol (from dates) at room temperature. Juice mixing with bioethanol showed glucose content was stable for 2 days and from 3 days it was started to rot slowly and bioethanol production was lower than control. It might be due to the bioethanol effects on the juice as solvent that is why rotten started in the juice about 2 days earlier and glucose content reducing as well as grapes bioethanol production (in rotten condition) made faster in the control (without bioethanol) than in the juice with date bioethanol. From this results it can be suggested that bioethanol also can be used as antiseptic effects on sanitization and laboratory use.

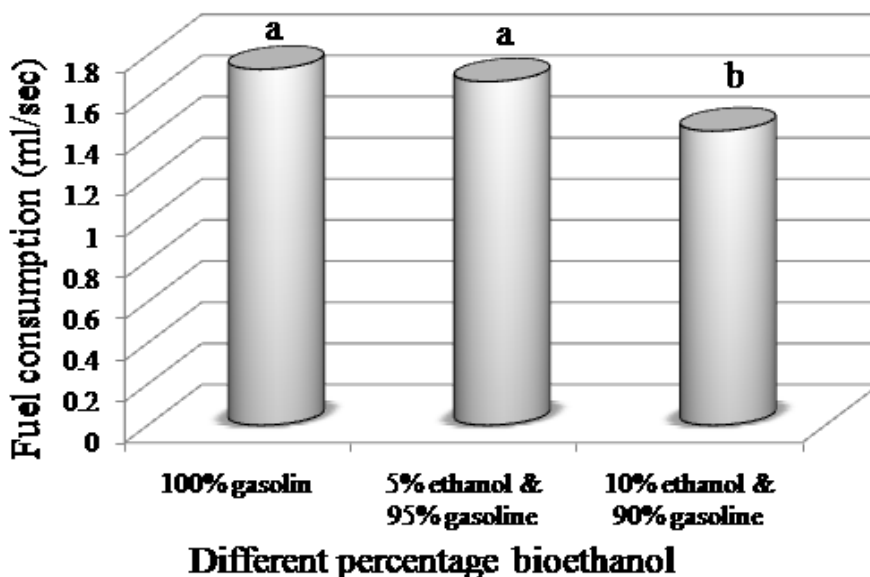


Fig. 10. Fuel consumption estimation using 2 stroke engine at pure gasoline (100%), 5% bioethanol +95% gasoline and 10% bioethanol + 90% gasoline. different letters mean the significance by LSD (p=0.05)

Table 1. Determination of viscosity and acid value bioethanol produced at different days of fermentation. Mean± Se (n =3)

Properties	Days			ASTM standard of viscosity and acid value
	1	2	3	
Viscosity (cst)	2.1±0.2	2.0±0.4	1.8±0.1	1.9 - 6.0
Acid value (mg KOH/g)	0.5	0.4	0.3	0.0 - 0.5

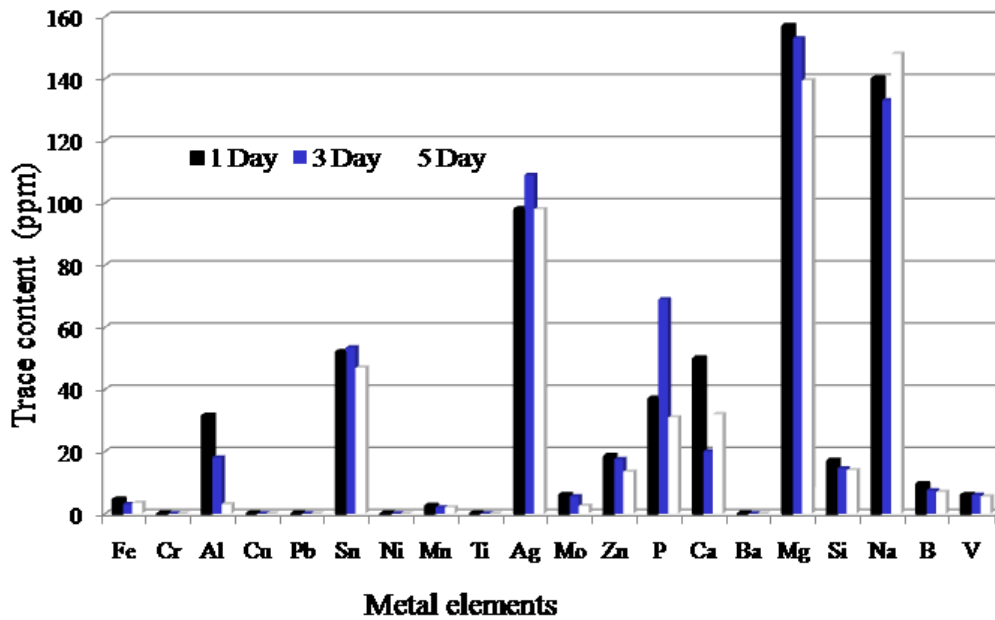


Fig. 11. Metals content of bioethanol produced at different days of fermentation

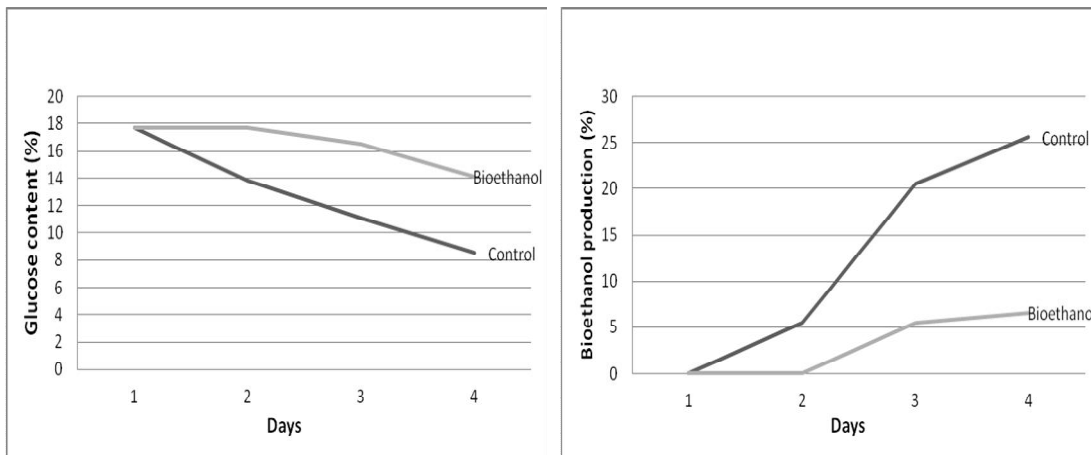


Fig. 12. Determination of glucose content and bioethanol production at different days using bioethanol as solvent

4. CONCLUSIONS

From the study, it can be concluded that bioethanol can be produced from waste dates as the substrates. The best yield (22.5%) of bioethanol was found in different parameters such as 5.8 pH, 28°C temperatures, 5 days fermentation period and 4 g/l yeast (*S. cerevisiae*) concentration. The bioethanol fuel from the waste dates can be safe for use in the petrol engine as it did not contain any unwanted metal elements. Bioethanol showed lower fuel consumption and emissions namely CO₂, CO,

SO_e, HC and NO_x compared to the pure petrol fuel. The study suggests that bioethanol production from date wastes can help to clean the environment from wastes by recycling.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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